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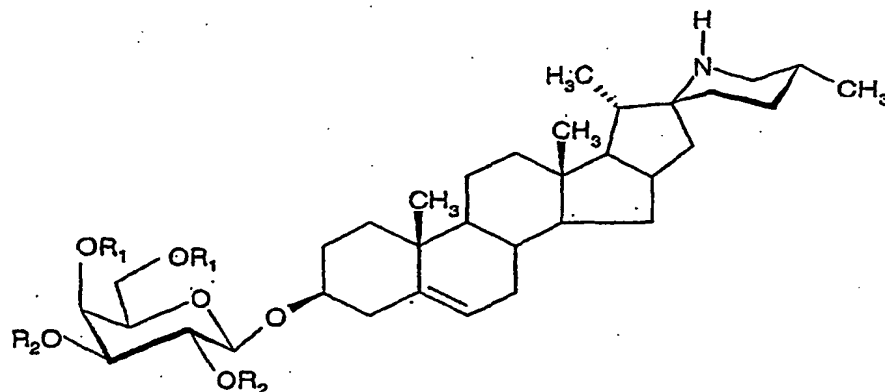
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(54) Title: SYNTHESIS OF SOLANUM GLYCOSIDES



(II)

(57) Abstract: The present invention relates to the chemical synthesis of solanum glycosides, in particular to the synthesis of solanone as well as to novel  $\beta$ -monosaccharide intermediate compounds, of Formula (II), where each  $R_1$  is the same or different and independently represents a benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene group and each  $R_2$  is the same or different and independently represents a benzoyl, acetyl or pivaloyl group.

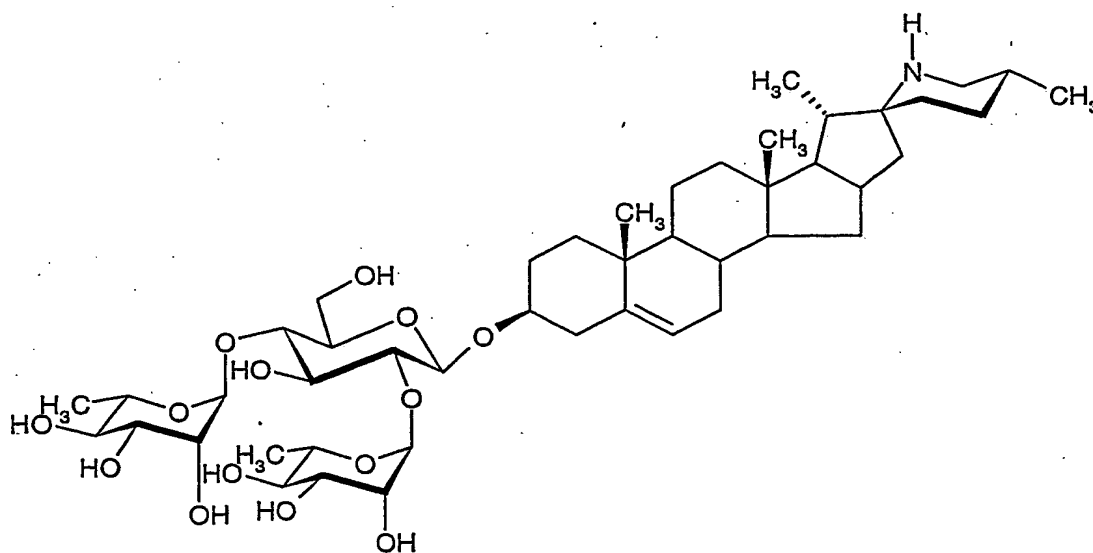
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## Synthesis of Solanum Glycosides

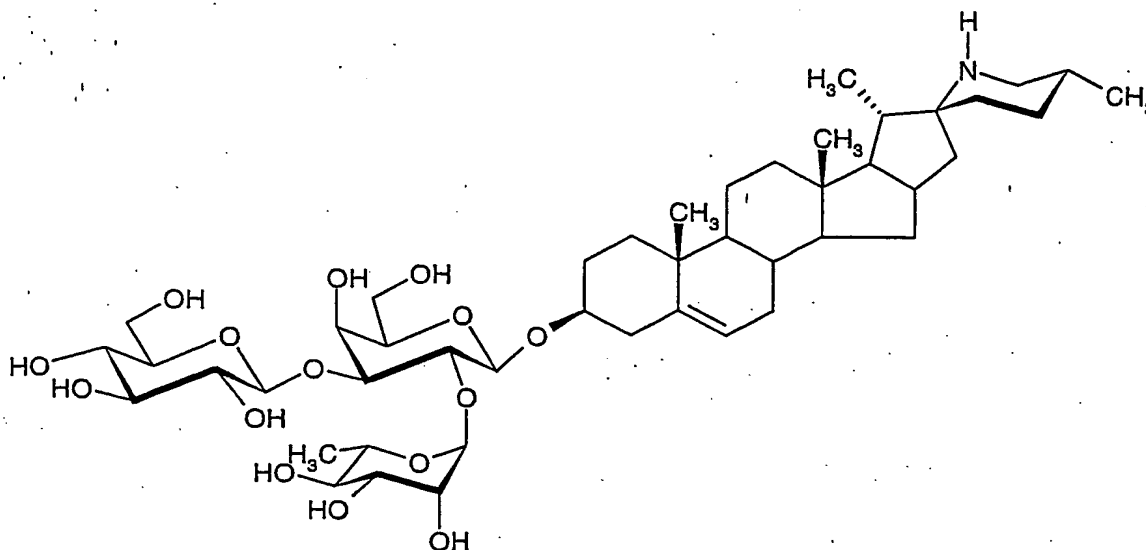
The present invention relates to the chemical synthesis of solanum glycosides, in particular to the synthesis of solasonine as well as to novel  $\beta$ -monosaccharide intermediate compounds.

Solasodine and its glycosides are of considerable interest clinically. They are widely used as starting products for the synthesis of various steroidal drugs, the aglycon solasodine is a source for synthetic cortisone and progesterone.

It is moreover well established that certain naturally occurring conjugate solasodine glycosides have potent antineoplastic properties. Of particular interest are the triglycosides solasonine (22R, 25R)-spiro-5-en-3 $\beta$ -yl- $\alpha$ -L-rhamnopyranosyl-(1->2 gal)-O-p-D-glucopyranosyl-(1->3 gal)- $\beta$ -D-galactopyranose and solamargine (22R, 25R)-spiro-5-en-3 $\beta$ -yl- $\alpha$ -L-rhamnopyranosyl-(1->2 glu)- $\alpha$ -L-rhamnopyranosyl- (1->4 glu)- $\beta$ -D-glucopyranose. The structures of these triglycosides are shown below:



Solamargine



Solasonine

The above triglycosides are conventionally obtained by extraction from a plant source. A commercially available extract of *S. sodomaeum*, commonly referred to as BEC (Drug Future, 1988, vol. 13.8, pages 714-716) is a crude mixture of solamargine, solasonine and their isomeric diglycosides. The extraction process for making BEC involves homogenizing the fruits of *S. sodomaeum* in a large volume of acetic acid, filtering off the liquid through muslin followed by precipitation of the glycosides with ammonia (Drugs of today (1990), Vol. 26 No. 1, p. 55-58, cancer letters (1991), Vol. 59, p. 183-192). The yield of the solasonine glycoside mixture is very low (approx. 1%). Moreover the individual process steps are not defined to GMP in terms of scale up, definition of yield, composition and product quality.

There is a great need for a cost efficient process that provides the antineoplastically active triglycoside solasonine at high yield with little or no impurities.

Contrary to other steroid ring systems, the steroid skeleton of solasonine contains a very labile nitrogen-containing ring. This aglycon cannot readily be chemically modified while keeping the steroid skeleton intact. In spite of the fact that the

aglycon solasodine is readily available, the prior art does not disclose the synthesis of the solasonine using the aglycon material as starting material.

The synthesis of solasonine requires the stereoselective glycosylation of solasodine at the relatively unreactive hydroxyl group.

It has been found that solasodine is not compatible with the conventional steroid glycosylation technique. No glycosylation was observed following the treatment of solasodine with tetrabenzoyl  $\alpha$ -D-glucopyranosyl trichloroacetimidate and trimethyl-silyl triflate or boron trifluoride dietherate (unpublished results).

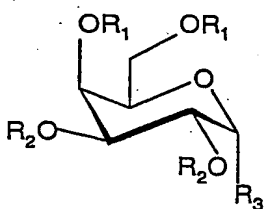
The problem underlying the present invention is to provide a cost effective method for the preparation of solasonine.

The present invention resides in the finding that the stereoselective  $\beta$ -glycosylation of solasodine may be achieved in high yields using specific galacto-pyranosyl donors. Preferably the reaction is carried out in the presence of a promoter.

### Detailed description of the invention

It was unexpectedly found that by reacting a D-galacto-pyranosyl donor of the following formula 1

Formula 1



$R_1$  = Benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene

$R_2$  = Benzoyl, acetyl or pivoyl

$R_3$  = Halogen, SEt or SPh

Formula 1

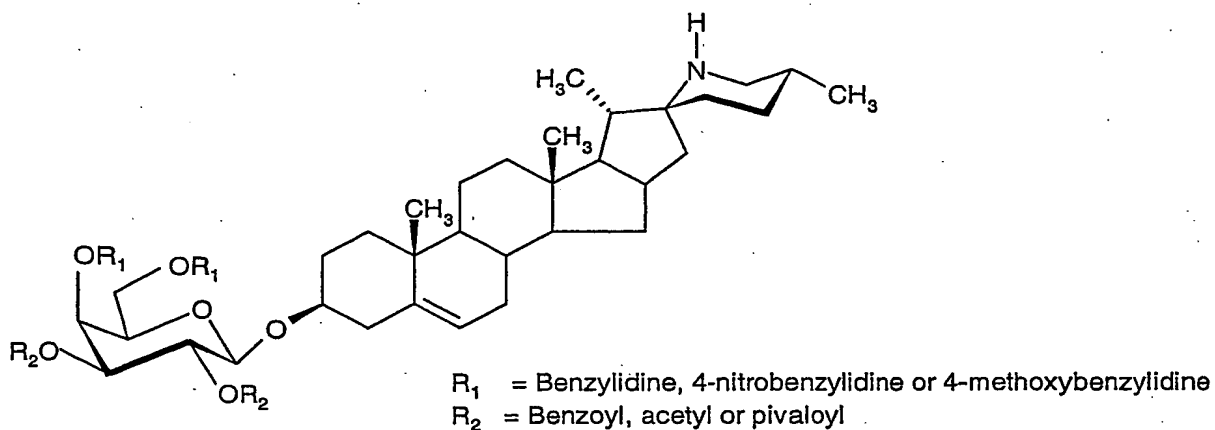
wherein each  $R_1$  is the same or different and independently represents a benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene group

each R<sub>2</sub> is the same or different and independently represents a benzoyl, acetyl or pivaloyl group and

R<sub>3</sub> is halogen, SPh or SEt

with solasodine the correspondingly protected  $\beta$ -glycoside of formula 2 could be obtained in high yield.

Formula 2



wherein each R<sub>1</sub> is the same or different and independently represents a benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene group and each R<sub>2</sub> is the same or different and independently represents a benzoyl, acetyl or pivaloyl group.

Preferably the reaction is carried out in the presence of a promoter.

Any conventional promoter as used in carbohydrate chemistry may be used.

The following promoters are particularly preferred:

Silver triflate, boron trifluoride diethyl etherate (-10°C), trimethylsilyl triflate bromide, N-iodosuccinimide, thiomethyl sulfonium triflate.

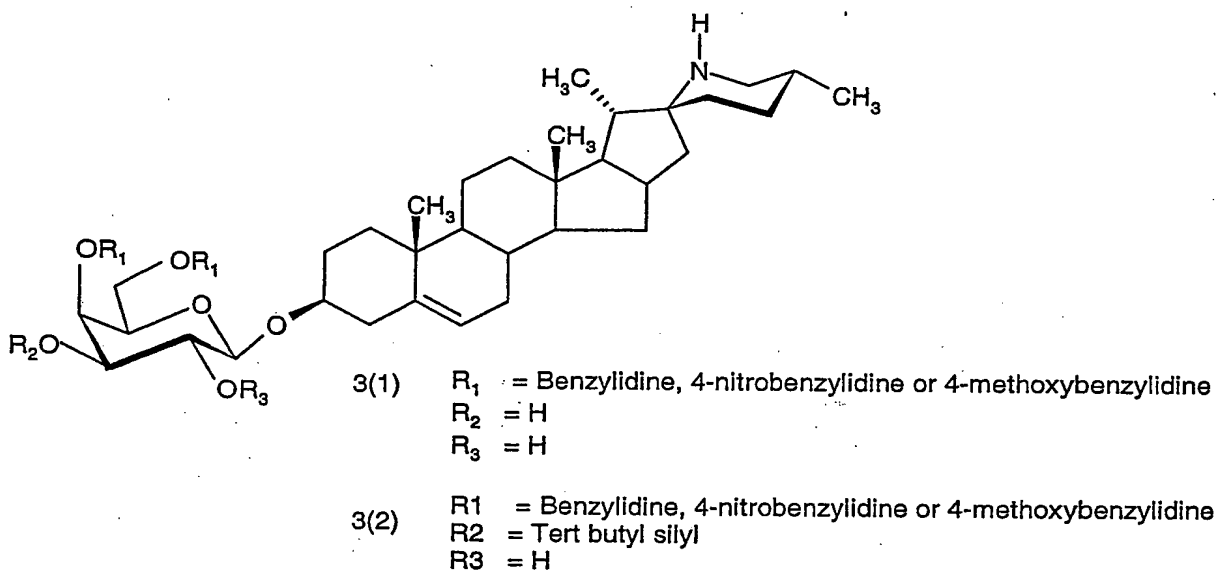
The reaction is preferably carried out using dichloromethane as the solvent. Preferably the reaction time is 30 min.-1 hr.

The desired end product solasonine may be prepared by partially deprotecting the  $\beta$ -glycoside of formula 2 to give intermediate formula 3(1) and then selectively

silylating one of the hydroxyl groups (OH-2 and OH-3) using tert-butyldimethylsilyl chloride, imidazole in DMF at 50°C.

However, due to the small selectivity between the OH-2 and OH-3 hydroxyl groups a mixture of OH-2 and OH-3 silylated protected  $\beta$ -glycosides are formed. The OH-3 silylated protected  $\beta$ -glycoside can mostly be precipitated from the mixture in methanol of the formula 3(2).

### Formula 3



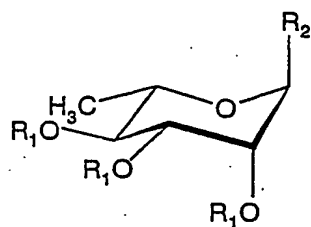
wherein each of  $R_1$  is the same or different and represent independently from each other benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene,

$R_2$  is tert-butyldimethylsilyl or H and  $R_3$  is H.

The OH-3 protected galactose-solasodine adduct is then glycosylated at the OH-2 with a suitable  $\alpha$ -L-rhamnopyranosyl donor.

Suitable rhamnose donors include tri-O-benzoyl- $\alpha$ -rhamnopyranosyl, tri-O-pivaloyl-L-rhamnopyranosyl, or tri-O-acetyl- 4- L-rhamnopyranosyl bromides of formula 4

### Formula 4



$R_1$  = Acetyl, benzoyl or pivaloyl

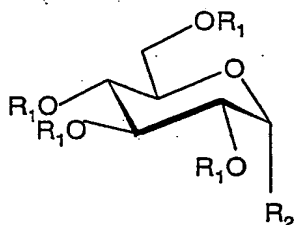
$R_2$  = Halogen, SEt or SPh

wherein each of  $R_1$  is the same or different and independently represents acetyl, benzoyl or pivaloyl,  
and  $R_2$  is halogen or SEt, SPh

Deprotection of the tert-butyldimethylsilyl group at the OH-3 position using tetrabutylammonium fluoride in THF and glycosidation with a suitable  $\alpha$ -D-glucopyranosyl donor,

wherein the D-glucopyranosyl donor is tetra-O-benzoyl- $\alpha$ -D-glucopyranosyl bromide, tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide or tetra-O-pivaloyl- $\alpha$ -D-glucopyranosyl bromide, or a thio-glycoside of the general formula 5.

#### Formula 5

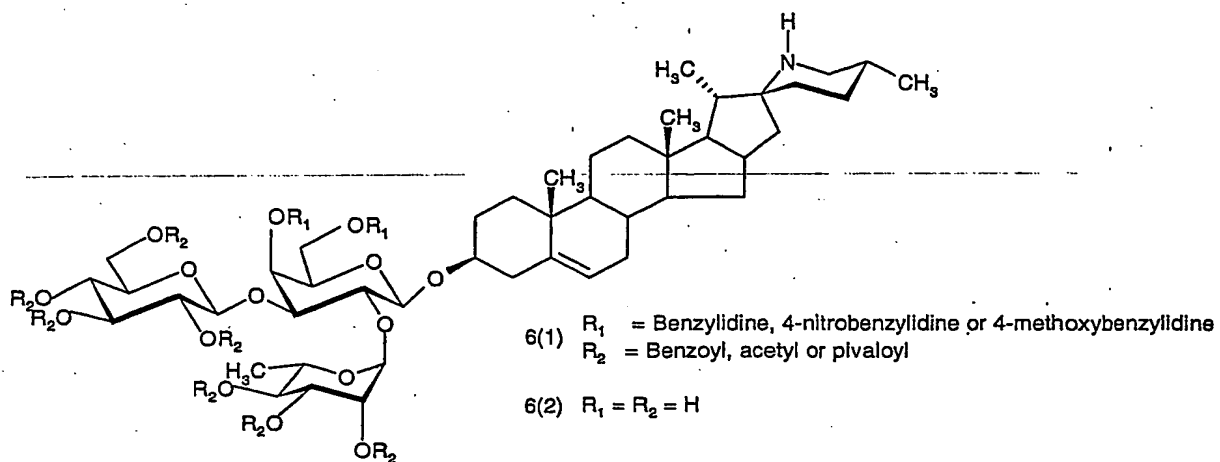


$R_1$  = Acetyl, benzoyl or pivaloyl

$R_2$  = Halogen, SEt or SPh

wherein each of  $R_1$  is the same or different and independently represents acetyl, benzoyl or pivaloyl and  $R_2$  is halogen, SEt, SPh gives a fully protected solasonine of formula 6(1).

## Formula 6



wherein each  $R_1$  is the same or different and independently represents benzyldiene, 4-nitrobenzyldiene or 4-methoxybenzyldiene and each  $R_2$  are the same or different and independently represent acetyl, benzoyl or pivaloyl.

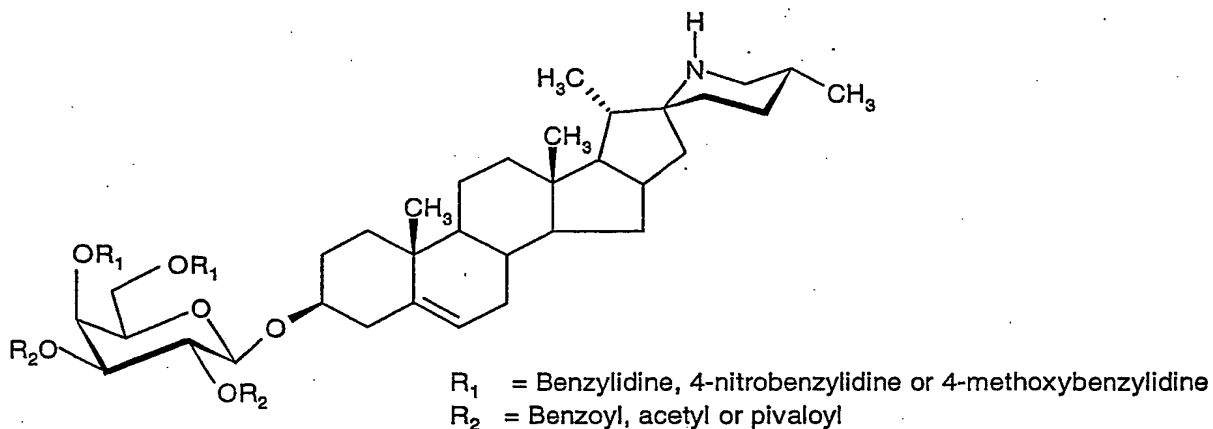
The protected solasonine formula 6(1) may be de-acetalised using aqueous acetic acid at 70°C and de-esterified using sodium methoxide in methanol/dichloromethane mixture to give the fully deprotected solasonine formula 6(2) where in  $R_1$  and  $R_2$  are H.



## Claims

1. A galactose-solasodine conjugate of the general formula 2 or a derivative thereof

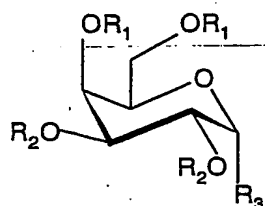
Formula 2



wherein each  $R_1$  is the same or different and represents benzylidene, 4-nitro benzylidene or 4-methoxybenzylidene and each  $R_2$  is the same or different and represents benzoyl, acetyl or pivaloyl.

2. A method for the preparation of the galactose-solasodine conjugate as defined in claim 1, comprising the reaction of solasodine with a galactopyranosyl donor of general formula 1

### Formula 1



R<sub>1</sub> = Benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene.

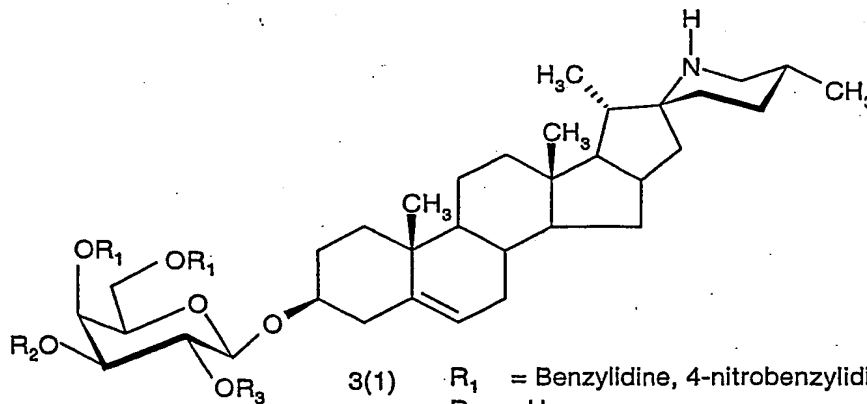
$R_2$  = Benzoyl, acetyl or pivoyl

$R_3$  = Halogen, SEt or SPh

wherein each R<sub>2</sub> independently represents a benzoyl, acetyl or pivaloyl group, and R<sub>1</sub> is benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene and R<sub>3</sub> is halogen, SE or SPh .

3. A method for the preparation of solasonine comprising the silylation of the diol of formula 3(1) to give a selectively silylated product in the OH-3 position 3(2)

### Formula 3

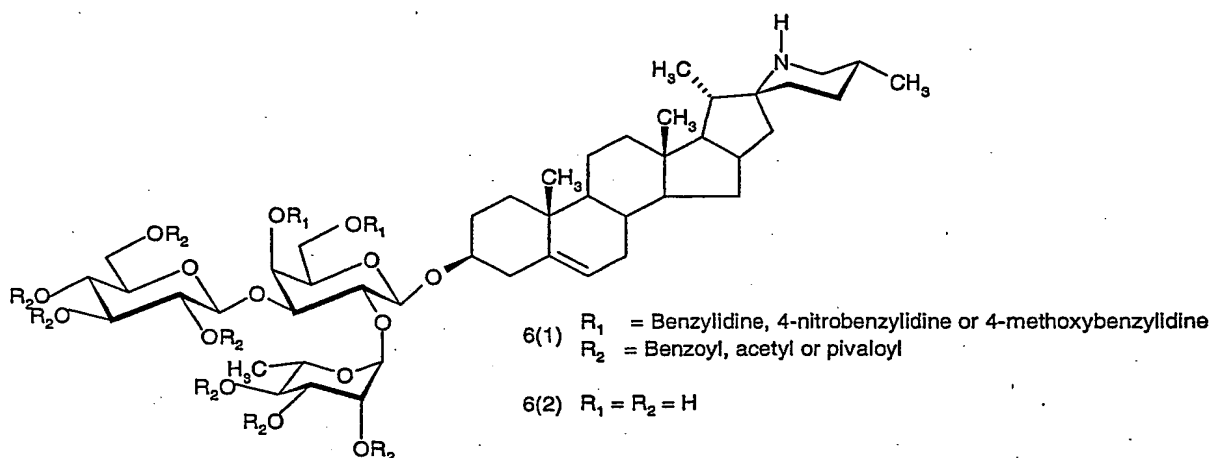


3(1)  $R_1$  = Benzylidene, 4-nitrobenzylidene or 4-methoxybenzylidene  
 $R_2$  = H  
 $R_3$  = H

3(2) R1 = Benzyldiene, 4-nitrobenzyldiene or 4-methoxybenzyldiene  
R2 = Tert butyl silyl  
R3 = H

wherein  $R_1$  represents either benzylidene, 4-nitrobenzylidene, or 4-methoxybenzylidene acetal protecting group by glycosylation of OH-2 with an  $\alpha$ -L-rhamnopyranosyl donor, followed by deprotection of the group on the hydroxyl (OH-3) and a further glycosylation of the hydroxyl (OH-3) with  $\alpha$ -D-glucopyranosyl donor to yield the protected solasonine of formula 6(1) which is de-acetalised and de-esterified to yield a solasonine of formula 6(2).

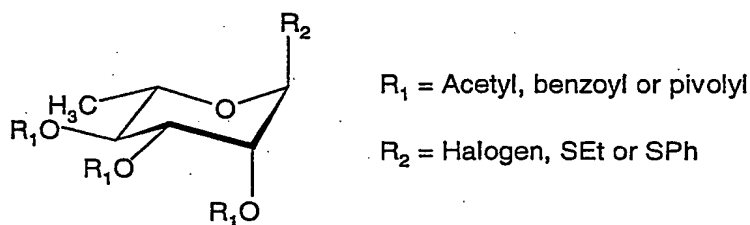
Formula 6



4. The method according to claim 3, wherein the D-gluco-pyranosyl donor is tetra-O-benzoyl- $\alpha$ -D-glucopyranosyl bromide, tetra-O-acetyl- $\alpha$ -D-glucopyranosyl bromide or tetra-O-pivaloyl- $\alpha$ -D-glucopyranosyl bromide.
5. The method according to claim 2-4, wherein the glycosylation reaction is carried out in the presence of a promoter selected from silver triflate, boron trifluoride diethyl etherate, trimethylsilyl triflate bromide, N-iodosuccinimide or dimethyl thiomethyl sulfonium triflate.

6. The method of claim 3, wherein the protected glycoside is deprotected in methanol-dichloromethane solution by treatment with sodium methoxide, followed by neutralization with a mild acid ion-exchange resin.
7. The method of claim 3-6, wherein the hydroxyl groups (OH-4 and OH-6) are protected by acetalisation with Benzaldehyde dimethoxy acetal in DMF and a catalytic amount of para-toluene sulphonic acid.
8. The method of claim 3-7, wherein the rhamnose donor is tri-O-benzoyl- $\alpha$ -L-rhamnopyranosyl bromide, or a thio-glycoside of the general formula 4

Formula 4

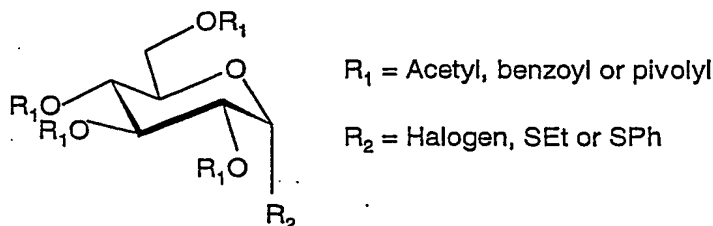


wherein each of the  $R_1$  is the same or different and each independently represent benzoyl, acetyl or pivaloyl and

$R_2$  is halogen, SEt or SPh.

9. The method of claim 3-8, wherein the glucose donor is tetra-O-benzoyl- $\alpha$ -D-glucopyranosyl bromide, or a thio-glycoside of the general formula 5

Formula 5



wherein each of  $R_1$  is the same or different and independently represents benzoyl, acetyl or pivaloyl and  $R_2$  is SEt, SPh or halogen

10. The method of claim 3-9, wherein the protected solasonine is de-acetalised and de-esterified by treatment with 80% acetic and then sodium methoxide solution in methanol-dichloromethane, followed by neutralization mild acid ion-exchange resin.

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/EP2004/004629

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C07J43/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEILSTEIN Data, CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FEWELL A M ET AL: "INTERACTIONS BETWEEN THE GLYCOALKALOIDS SOLASONINE AND SOLAMARGINE IN RELATION TO INHIBITION OF FUNGAL GROWTH" PHYTOCHEMISTRY, PERGAMON PRESS, GB, vol. 37, no. 4, 1994, pages 1007-1011, XP008011328 ISSN: 0031-9422 page 1010, left hand col., last paragraph	1-10
Y	WO 03/018604 A (GLYCOMED SCIENCES LTD ; SHAHID MOHAMMED (GB)) 6 March 2003 (2003-03-06) Step C, E, F  ----- -/--	1-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

12 August 2004

Date of mailing of the international search report

25/08/2004

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# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP2004/004629

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>LI B ET AL: "An improved synthesis of the saponin, polyphyllin D"  CARBOHYDRATE RESEARCH, ELSEVIER SCIENTIFIC PUBLISHING COMPANY. AMSTERDAM, NL,  vol. 331, no. 1,  9 March 2001 (2001-03-09), pages 1-7,  XP004317141  ISSN: 0008-6215  Scheme 1</p>	1-10
Y	<p>L. F. AWAD ET AL.: "A synthesis of 3-O-(alpha-D-mannopyranosyl)-D-mannose and its protein conjugate"  CARBOHYDRATE RESEARCH,  vol. 122, no. 1, 1983, pages 69-79,  XP002292126  example 12</p>	1-10
Y	<p>A. FÜRSTNER ET AL.: "Ring-closing alkyne metathesis. Application to the total synthesis of sophorolipid lactone"  J.ORG.CHEM.,  vol. 65, 2000, pages 8758-8762,  XP002292185  example 16</p>	1-10
Y	<p>FIGUEROA-PEREZ S ET AL: "Synthesis of a sialyl-alpha-(2-&gt;6)-lactosamine trisaccharide with a 5-amino-3-oxapentyl spacer group at C-1&lt;I&gt;"  CARBOHYDRATE RESEARCH, ELSEVIER SCIENTIFIC PUBLISHING COMPANY. AMSTERDAM, NL,  vol. 317, no. 1-4,  30 April 1999 (1999-04-30), pages 29-38,  XP004179992  ISSN: 0008-6215  examples 8-10</p>	1-10
A	<p>SUGIYAMA S DIAKUR J M: "A convenient preparation fo glycosyl chlorides from Aryl/Alkyl thiogl"  ORGANIC LETTERS, ACS, WASHINGTON, DC, US,  vol. 2, no. 17, 2000, pages 2713-2715,  XP002956970  ISSN: 1523-7060  examples 25-28</p>	1-10

### Information on patent family members

PCT/EP2004/004629

Form PCT/ISA/210 (patent family annex) (January 2004)